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(54) Title: <b>POLYMER CARRIER FOR CULTIVATION OF KERATINOCYTES</b>			
(57) Abstract  A polymer carrier for keratinocyte cultivation on biologically active polymer bases, prepared by radical polymerization of a polymerization mixture containing 1-95 wt.% of a radical-polymerizable monomer, 0.0-10 wt.% of a crosslinker, 0.1-5 wt.% of an initiator, 0.0-60 wt.% of a solvent, 0.0-50 wt.% of a polymerizable derivative of a sterically hindered amine, 0.0-60 wt.% of a polymerizable saccharide derivative, and polymerizable derivatives of reactive $\omega$ -acryloyl- or methacryloyl amino acids, which can be used for additional modification of polymer carriers with appropriate saccharide or sterically hindered amine derivatives. A hydrophilic polymer carrier with no bonded polymerizable saccharide or sterically hindered amine derivatives can be activated by sorption of specific derivatives of biologically active substances on the carrier surface.			

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## Description

### Polymer carrier for cultivation of keratinocytes

#### 5    Technical Field

The invention relates to the polymer carrier for cultivation of keratinocytes on biologically active polymer bases.

#### Background Art:

10        Keratinocytes are commonly utilized for covering large skin defects such as burns, trophic-ulcers, and bed-sores. A small piece of skin is taken from a patient (about 3 sq. cm), from which keratinocytes are isolated enzymatically. These are then cultivated under the conditions of tissue cultures together with mouse 3-T-3 fibroblasts (so-called feeder cells), in which proliferation was stopped by  $\gamma$ -irradiation or  
15    chemically. Human keratinocytes cannot adhere to the cultivation vessel without these feeder cells. After extinction of feeder cells and proliferation of keratinocytes, the latter are enzymatically released from the bottom of the cultivation vessel, attached to a greasy tulle and transferred to skin defects (Green et al., Proc. Natl. Acad. Sci. USA 76, 5665-5668, 1979). Using this procedure, large areas of the damaged skin can be covered from  
20    a relatively small biopsy.. But the transfer proper of cells onto a greasy tulle is technically demanding and is frequently the cause of failure. Therefore a technology was prepared issuing from the Green method, which makes it possible to cultivate keratinocytes directly on polymer carriers and transplant them directly with the carriers onto the wound area (Vacík et al., Czech Patent 281 269, 1996; Dvořánková et al., Folia Biol., Praha, 42, 83-86, 1996; Smetana et al., J. Mater. Sci. Mater. Med. 8, 587-590,  
25    1997; Dvořánková et al., Biomaterials, V. 19 , 141-146 (1998).

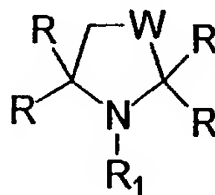
Polymer carriers are prepared by radical polymerization under the conditions common for these types of polymerizations. The prepared carriers are additionally purified by washing out perfectly the residues of unreacted monomers or the used  
30    solvent. So far, polymer carriers of poly(HEMA) material, prepared by radical polymerization of 2-hydroxyethyl methacrylate (HEMA), have been used. Before

application of cells, it is necessary to preincubate the carrier for 24 h in a medium containing 25 % of bovine serum. The keratinocyte cultivation proceeds in the presence of feeder cells under the conditions of tissue cultures, hence together with mouse 3-T-3 fibroblasts, the proliferation of which was stopped by  $\gamma$ -irradiation or chemically. The procedure also complicates the whole transplantation process continuing to be connected with necessary preincubation and the presence of feeder cells.

A significant shortcoming is also the fact that the presence of feeder cells from the cultivation system loads the patient's immunological system and that the carrier prepared in this way does not possess properties which would suppress the formation of free radicals or reactive oxygen products, which makes the healing of the affected tissue difficult.

#### Summary of Invention

The substance of the invention, which eliminates the above-mentioned shortcomings, is the polymer carrier for cultivation of keratinocytes prepared by radical polymerization of a polymerization mixture which contains 1-95 wt.-% of radical-polymerizable monomers, 0.0-10 wt.-% of a crosslinker, 0.0-10 wt.-% of an initiator, 0.0-60 wt.-% of a solvent, 0.0-60 % of polymerizable saccharide or disaccharide derivatives, 0.0-50 wt.-% of polymerizable  $\alpha$ -amino acid derivatives or their reactive derivatives and 0.0-50 wt.-% of polymerizable derivatives of sterically hindered amine of general formula



where W is -CH(X)- or -CH(X)CH<sub>2</sub>- and X is a radical-polymerizable group.

Keratinocytes can be cultivated on such bases prepared in this way without any prior modification.

The object of the invention is further developed by pointing to suitable compounds and their combinations and procedures.

The polymer carrier prepared in the absence of polymerizable saccharide derivatives is further conditioned by adsorption of biologically active saccharides selected from the group of polysaccharides heparin (A), heparan sulfate (B), hyaluronic acid (C), further monosaccharides conjugated with albumin or with a polymer carrier  
5 such as glucuronic acid (D),  $\beta$ -D-galactose (E),  $\beta(\alpha)$ -D-N-acetylgalactosamine (F),  $\beta(\alpha)$ -D-glucosamine (G),  $\beta$ -D-mannose (H), where the adsorption proceeds at a concentration of 10-500  $\mu$ g/ml of PBS (phosphate-buffered saline) at temperatures 4-37° C for 1-12 h.

The invention is based on the new finding that with certain properties of the  
10 polymer carrier, it is possible to cultivate keratinocytes in the absence of auxiliary cells on polymers with biologically active polysaccharides, neoglycoproteins and neoglycoligands adsorbed on a synthetic polymer carrier. In further applications of the polymer carrier as an optimum cover for transplanted cells, the presence of chemically bonded sterically hindered amine derivatives plays an important role. These amines  
15 preferentially react with oxygen and its reduced derivatives, such as superoxide, hydroxy radical, hydrogen peroxide, dialkyl peroxides, alkylhydroperoxides, and hence prevent the destructive oxidation of the living tissue. Thus, in contact with the living tissue, they have the ability to liquidate, in a pronounced way, reactive oxygen products and to contribute to acceleration of healing of damaged tissues.

20 In polysaccharide adsorption, they are used in a native form or with bonded biotin, which facilitates their adsorption. Monosaccharides are used in the form of neoglycoproteins of the general structure: monosaccharide - bovine serum albumin with or without biotin (Lee and Lee in *Lectins and Glycobiology*, H.-J. Gabius and S. Gabius, Eds, Springer Laboratory, Berlin 1993, pp 9-22; Gabius et al., *Histol. Histopathol.* 8, 369-383, 1993) or in the form of neoglycoligands: monosaccharide - poly[N-(2-  
25 hydroxyethyl)acrylamide] with or without biotin (Bovin in *Lectins and Glycobiology*, H.-J. Gabius and S. Gabius, Eds, Springer Laboratory, Berlin 1993, pp 23-28; Bovin et al., *Chem. Soc. Rev.* 24, 413-321, 1995).

The given polysaccharides or monosaccharide derivatives are immobilized on  
30 the polymer carrier surface by adsorption of individual substances or their combinations (e.g., B:F 1:1, B:F:H 2:1:1). It is proceeded as follows. The polymer carrier is incubated,

under sterile conditions, with a polysaccharide (A-C), neoglycoprotein or neoglycoligand (D-H) or their mixture in concentration of 10-500  $\mu\text{g}/1\text{ ml}$  PBS (phosphate-buffered physiological saline) for 1-12 h. An excess solution is removed, the carrier is carefully rinsed with a cultivation medium and keratinocytes are applied at a  
5 density of  $1-5 \times 10^6$  per  $50\text{ cm}^2$  area. Cells are cultivated at  $37^\circ\text{C}$  in the atmosphere of 3.3 % of  $\text{CO}_2$ . In this way, both the keratinocytes from an enzymatically released, fine dermo-epidermal graft taken from the patient and those from frozen cells can be cultivated.

The proliferated keratinocytes on the polymer carrier can be transferred onto a  
10 skin defect and used for its covering. At that, the carrier with cells is oriented in such a way that the cells are applied onto the wound area and the polymer carrier forms an optimum cover of transplanted cells. In this way, both autologous (patient's own cells) cells, forming a permanent cover, and allogenic cells (of another human), which pronouncedly stimulate the healing, can be applied. If the polymer carrier is prepared  
15 using polymerizable saccharide derivatives, the preincubation of the carrier proper with polysaccharides, neoglycoproteins or neoglycoligands can be obviated. In addition, if covalently bonded onto a polymer carrier, the concentration of saccharides can be exactly determined. Further procedure of cultivation is retained.

By eliminating feeder cells from the cultivation system, the immunological  
20 loading of the patient is lower and the process of keratinocyte transplantation is simpler and more effective.

### Examples

#### 25 Example 1

A mixture of 100 g of 2-hydroxyethyl methacrylate, 0.4 g of ethylene dimethacrylate, 1.4 g of 2-hydroxy-2-methylpropiophenone, and 6 g of 2,2,6,6-tetramethylpiperidin-4-yl methacrylate was polymerized on a polypropylene film by 10 min irradiation with a series of 175-W UV lamps from a distance of 18 mm. A foil 1 mm thick was formed,  
30 which was extracted with a mixture of ethanol and water (1:1) for 48 h. The foil can be water-swollen to the water content of 36 %.

## Example 2

A mixture of 80 g of 2-hydroxyethyl methacrylate, 5 g of *N*-(2,2,6,6-tetramethylpiperidin-4-yl)methacrylamide, 0.6 g of ethylene dimethacrylate, 0.5 g of 2,2'-azobis(2-methylpropionitrile) is dosed, after bubbling with argon (10 min), under an inert atmosphere into molds suitable for preparation of films (thickness 1.3 mm) where it is polymerized at 70° C for 12 h. The resulting film is washed with 30% ethanol and water.

## 10 Example 3

A mixture of 40 g of 1-vinylpyrrolidin-2-one, 40 g of 2-acetoxyethyl methacrylate, 0.75 g of 1,1'-divinyl-3,3'-(ethane-1,1-diyl)di(pyrrolidin-2-one), 15 ml of glycerol, 2 g of 2-deoxy-2-methacryloylamino-D-galactopyranose, 2.5 g of 1,2,2,6,6-pentamethylpiperidin-4-yl methacrylate and 0.50 g of dimethyl 2,2'-azobis(2-methylpropionate) was bubbled with argon (10 min) and filled into molds for preparation of foils (foil thickness 1.1 mm) and polymerized for 12 h at 71° C. The foils were washed with 30 % ethanol and finally swollen in distilled water.

## Example 4

20 A mixture of 60 g of 2-hydroxyethyl methacrylate, 20 g of 2-(2-hydroxyethoxy)ethyl methacrylate, 3 g of 2-methacryloyloxyethyl 2,2,5,5-tetramethyl-1*H*-2,5-dihydropyrrole-3-carboxylate, 0.5 g of ethylene dimethacrylate, 3.5 g of 2-deoxy-2-[[6-(methacryloylamino) hexanoyl]amino]-D-glucopyranose, 0.5 g of 2,2'-azobis(propionitrile), 20 ml of poly(ethylene glycol) 300 (Macrogolum 300), after  
25 bubbling with argon (12 min) is dosed in an inert atmosphere into moulds for preparation of foils (thickness 1.6 mm) and polymerized at 72° C for 11 h. The copolymer was extracted at 25° C with a mixture of 30 % ethanol for 72 h. The resulting foils were swollen in distilled water.

## Example 5

A mixture of 40 g of 1-vinyl-2-pyrrolidone, 30 g of 2-acetoxyethyl methacrylate, 0.75 g of 1,1'-divinyl-3,3'-(ethane-1,1-diyl)di(pyrrolidin-2-one), 3.5 g of 4-nitrophenyl 10-(methacryloylamino)decanoate, 0.2 g of dimethyl 2,2'-azobis(2-methylpropionate).

- 5 After bubbling argon for 10 min, the reaction mixture was filled into moulds for foil preparation (1.6 mm thickness) and polymerized for 12 h at 71° C. The reaction with a 25-fold molar amount of D-glucosamine was performed after swelling the foil in dimethyl sulfoxide (2 days, laboratory temperature). After finishing the reaction, the foil was swollen in a 3 % solution of ammonia and finally in distilled water.

10

## Example 6

A mixture of 100 g of 2-hydroxyethyl methacrylate, 0.4 g of ethylene dimethacrylate, 2 g of 2-deoxy-2-methacryloylamino-D-galactopyranose, and 0.14 g of dimethyl 2,2'-azobis(2-methylpropionate) was polymerized in polymerization forms (foil thickness 2

- 15 mm) at 68° C for 12 h. The foil formed was extracted with a mixture of ethanol and water (1:1) for 48 h. The foil can be swollen in water to the water content of 36 %.

Foils from a mixture of HEMA and 2-(2-hydroxyethoxy)ethyl methacrylate were prepared analogously.

## 20 Example 7

A mixture of 40 g of 1-vinylpyrrolidin-2-one, 40 g of 2-acetoxyethyl methacrylate, 0.75 g 1,1'-3,3'-(ethan-1,1-diyl)di(pyrrolidin-2-one), 0.15 g of dimethyl 2,2'-azobis(2-methylpropionate) was bubbled with argon (10 min), and filled into moulds for foil preparation (1.5 mm thickness) and polymerized for 12 h at 71° C. The foils were

25 washed with 30 % ethanol and finally swollen in distilled water.

## Example 8



The carrier prepared according to Examples 6 or 7 in the form of a disk, square or net 25-100 in diameter was placed on the bottom of a cultivation vessel and preincubated with polysaccharides or neoglycoligands (A-H) alone or in combination (e.g., B:F 1:1, B:F:H 2:1:1) in concentration 10-500 g/ml PBS for 1-12 h. Human keratinocytes were applied onto a carrier in density of  $4 \times 10^4/\text{cm}^2$ . The cells were cultivated at 37° C in the atmosphere of 3.3 % CO<sub>2</sub>. Keratinocytes for cultivation are obtained by taking from a patient a fine dermo-epidermal graft of 0.2-0.3 mm thickness, from which keratinocytes were obtained by treatment with trypsin.

The cultivation medium contained:

- 1) Eagle-H MEM with nonessential amino acids and sodium pyruvate (Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague)
- 2) glutamine (Institute for Serums and Vaccination Compounds, Prague), 0.3 mg/ml
- 3) 10 % bovine serum (ZVOS Hustopeče)
- 4) hydrocortison (Spofa Comp., Prague), 0.5 µmol/ml
- 5) penicillin (200 units/ml), BIOTIKA, Slovak Republic
- 6) gentamicin (0.16 mg/ml), lek. Slovenia
- 7) insulin (Actrapid MC NOVO, Denmark), 0.12 I.U/ml
- 8) cholera toxin (Sigma, Prague),  $10^{-10}$  M
- 9) epidermal growth factor (Sigma, Prague), 10 ng/ml.

## Example 9

### Evaluation of polymer carriers

Orientation experiments with keratinocyte cultivation were performed on various polymer carriers, whose composition is given in the following table. The carriers were prepared by radical polymerization using thermal or light initiators.

The polymerization using a thermal initiator was carried out after mixing and bubbling the polymerization mixture (argon, 10 min) between polypropylene plates at 70° C for

12 h (foil thickness 1.2 mm). The foils were extracted with 30 % ethanol for 3 days and with distilled water for 3 days.

Polymerization using UV initiation was performed in such a way that the polymerization mixture, after mixing, was poured onto a polyester base, where it was irradiated with  
5 175-W UV lamps from a distance of 15 cm for 12 min. The foils were washed with 30 % ethanol (2 days) and with distilled water (3 days).

The composition of polymerization charges for preparation of various polymer carriers is given in the following table 1.

10 a. The hydrophilic monomers used:

1. 2-hydroxyethyl methacrylate
2. (2-hydroxyethoxy)ethyl methacrylate
3. 1-vinylpyrrolidin-2-one
4. 2-acetoxyethyl methacrylate
- 15 5. methacrylic acid

b. Crosslinkers:

11. ethylene dimethacrylate
12. 1,1'-(ethane-1,1-diyl)di(pyrrolidin-2-one)

20

c. Polymerizable saccharides:

- A. 2-deoxy-2-[[6-(methacryloylamino)hexanoyl]amino]-D-glucopyranose
- B. 2-deoxy-2-methacryloylamino-D-galactopyranose
- C. 2-methacryloyloxyethyl 2-acetamino-2-deoxy-D-glucopyranoside
- 25 D. 6-deoxy-6-methacryloylamino-D-glucopyranose

d. Polymerizable sterically hindered amines:

I. 4-methacryloylamino-2,2,6,6-tetramethylpiperidine

II. (1,2,2,6,6-pentamethylpiperidin-4-yl) methacrylate

5 III. *N*-(2,2,5,5-tetramethylpyrrolidin-3-yl)methacrylamide

IV. 1,2,2,6,6-pentamethylpiperidin-4-yl 6-(methacryloylamino)hexanoate

e. Polymerization type - initiator used

- thermal polymerization: initiator dimethyl 2,2'-azobis(2-methylpropionate)

10 - UV polymerization initiator 2-hydroxy-2-methylpropiophenone

Solvent: glycerol (denoted G and amount in wt.-% given)

Poly(ethylene glycol 300 (Macrogolum 300) (denoted M and amount in wt.-% given)

Table 1.

E.N.	Monomer					Cross.		Saccharide				Amine				Polymerization		
	1	2	3	4	5	11	12	A	B	C	D	I.	II.	III.	IV.	T.	L	S.
1	99.5					0.3										0.2		
2	98.5					0.3											1.3	
3	68.5	30			1	0.3										0.2		
4	96.5					0.3										0.2		
5	65.6	30				0.3						3				0.2		
6			50	36.5			0.3					3					1.1	M10
7	96.5					0.3								3		0.2		G10
8	86.5				1	0.3		2										
9			50	36			0.3		3							0.2		G10
10	63.5	25			5	0.3				1						0.2		G10
11	86.5					0.3					3					0.2		G10
12	84.5					0.3					3	2				0.2		M10
13	85.5					0.3		2				2				0.2		G10

Remarks: E.N. Number of Experiment, Cross.: Crosslinker

Polymerisation: T-thermal, L-light, S-solvent

Note: the numbers given in the table are weight % relative to the polymerization mixtures.

f. Evaluation

The polymers prepared under the experiment Nos. 1-3 are standard polymer carriers which served as reference polymer bases. Human keratinocytes grow on these polymer bases after preincubation with bovine serum in the presence of mouse  
5 fibroblasts or after adsorption of bioactive saccharides in the absence of mouse fibroblasts.

Adhesion of human keratinocytes to polymer bases under the experiment Nos. 4-7 is markedly better in comparison with reference bases (1-3). However, also in this case, activation of the base using sugars is necessary.

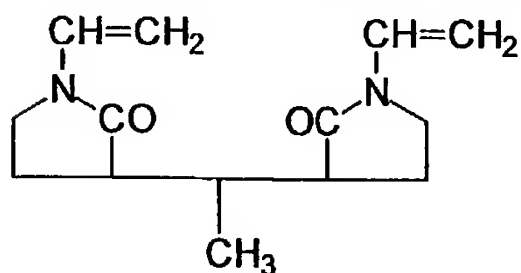
10 The results of cultivation of human keratinocytes on polymer bases Nos. 8-11 are very good. Keratinocytes adhere and grow without prior preincubation with bioactive polysaccharides. Base No. 10 appears the best. The results of cultivation on bases Nos. 12 and 13 were very close to cultivation results on base No. 10.

15 Industrial Applicability

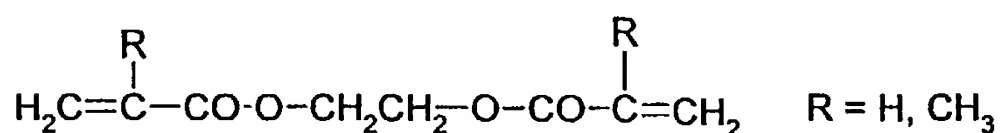
The polymer carrier for cultivation of keratinocytes on biologically active polymer bases can be utilized primarily for covering and, at the same time, treatment of large skin defects such as burns, trophic-ulcers and bed-sores.

### Claims

1. A polymer carrier for keratinocyte cultivation prepared by radical polymerization of a polymerization mixture containing 1-95 wt.-% of polymerizable monomers, 0.0-10 % wt.-% of a crosslinker, 0.0-10 wt.-% of an initiator, 0.0-60 wt.-% of a solvent, 0.0-60 wt.-% of polymerizable saccharide or disaccharide derivatives, 0.0-50 wt.-% of polymerizable sterically hindered amine derivatives, 0.0-30 wt.-% of polymerizable  $\alpha$ -amino acid derivatives or their reactive derivatives.
2. A hydrophilic polymer carrier for keratinocyte cultivation as claimed in claim 1, wherein the polymerization mixture contains 1-95 wt.-% of monomers, individual or in combination, selected from the group: acrylic and methacrylic acids, alkyl acrylates and methacrylates hydroxyalkyl acrylates and methacrylates, (alkyloxy)alkyl acrylates and methacrylates, (acyloxy)alkyl acrylates and methacrylates, acryl- and methacrylamides, (substituted alkyl)acrylamides and -methacrylamides, (hydroxyalkyl)acrylamides and -methacrylamides, 1-vinyl lactams, diacetoneacrylamide (1,1-dimethyl-3-oxobutyl)acrylamide.
3. A polymer carrier for keratinocyte cultivation prepared as claimed in claims 1 and 2 wherein the polymerization mixture contains 0.0-10 wt.-%, individual or in combination, of substances selected from the group: 1,1'-divinyl-3,3'-(ethane-1,1-diyl)di(pyrrolidin-2-one)

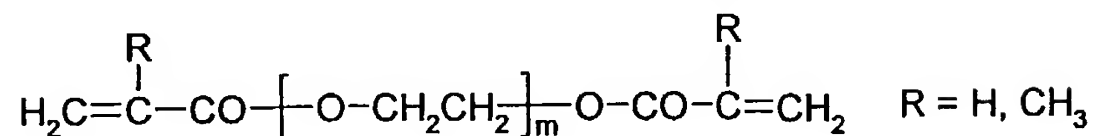


ethylene diacrylate, ethylene dimethacrylate



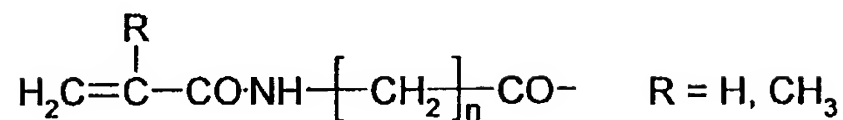
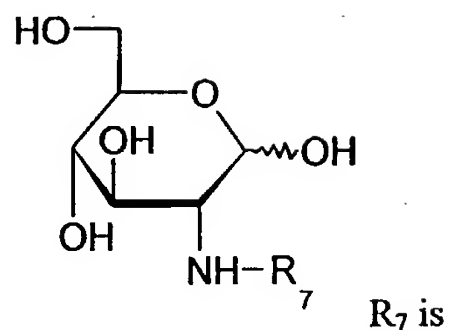
$\alpha$ -acryloyl- $\omega$ -acryloyloxypoly(oxyethylene)

$\alpha$ -methacryloyl- $\omega$ -methacryloyloxypoly(oxyethylene)



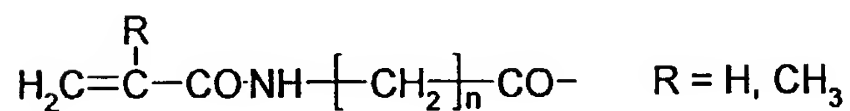
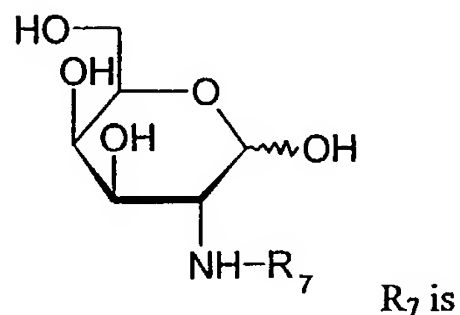
where m is 2-20.

4. A polymer carrier for keratinocyte cultivation prepared as claimed in claims 1-3  
 5 wherein it contains 0.1-10 wt.-% of substances, individual or in combination, selected from the group of substances, which undergo thermal decomposition thereby enabling a polymerization reaction, such as azodinitriles, azodiesteres, dialkylperoxides, diacylperoxides, or systems capable of initiating polymerization on irradiation with UV light or daylight such as benzoin  
 10 derivatives and  $\alpha$ -diketones (camphorquinone) in a mixture with tertiary amines.
5. A polymer carrier for keratinocyte preparation as claimed in claims 1-4 wherein it contains a solvent in an amount of 0.0-60 wt.-% and individually or in combination: ethylene glycol, di- and oligoethylene glycols, glycerol, ethylene  
 15 glycol and diethylene glycol mono- and diethers, 1-methylpyrrolidin-2-one, dimethylformamide, dimethylacetamide, dimethylsulfoxide.
6. A polymer carrier for keratinocyte preparation as claimed in claims 1-5 wherein polymerizable saccharide derivatives in amounts 0.0-60 wt.-% contain, individually or in combination polymerizable saccharide or disaccharide  
 20 derivatives selected from the group:
  - 2-deoxy-2-[[ $(n+1)$ -(acryloylamino)alkanoyl]amino]-D-glucopyranose
  - 2-deoxy-2-[[ $(n+1)$ -(methacryloylamino)alkanoyl]amino]-D-glucopyranose
  - 2-deoxy-2-[[6-(methacryloylamino)hexanoyl]amino]-D-glucopyranose
 for  $n = 5$ ,  $\text{R} = \text{CH}_3$



n is 1-10

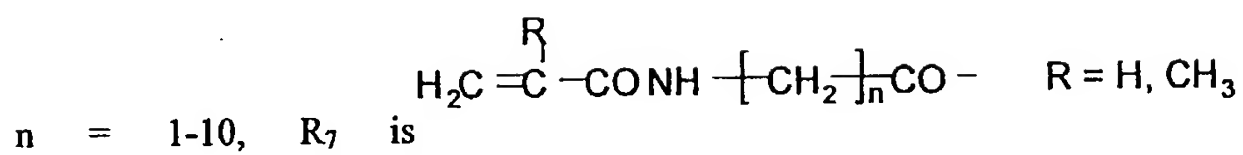
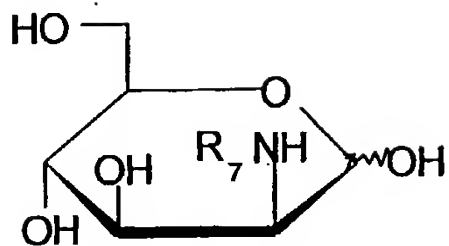
- 5      2-deoxy-2-{[(n+1)-(acryloylamino)alkanoyl]amino}-D-galactopyranose  
          2-deoxy-2-{[(n+1)-(methacryloylamino)alkanoyl]amino}-D-galactopyranose  
          2-deoxy-2-{[6-(methacryloylamino)hexanoyl]amino}-D-galactopyranose  
          for n = 5, R = CH<sub>3</sub>



R = H, CH<sub>3</sub>, n is 1-10

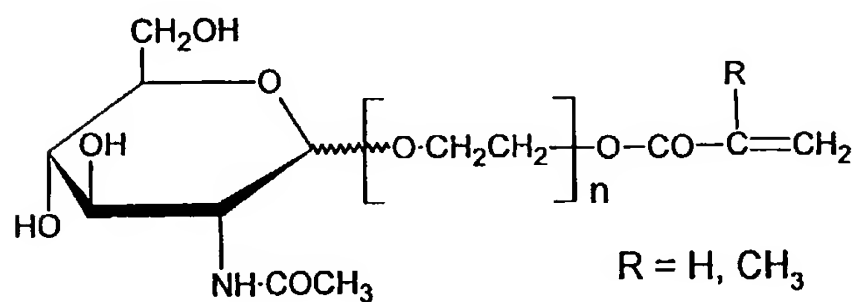
- 15      2-deoxy-2-{[(n+1)-(acryloylamino)alkanoyl]amino}-D-mannopyranose  
          2-deoxy-2-{[(n+1)-(methacryloylamino)alkanoyl]amino}-D-mannopyranose  
          2-deoxy-2-{[6-(methacryloylamino)hexanoyl]amino}-D-mannopyranose  
          for n = 5, R = CH<sub>3</sub>





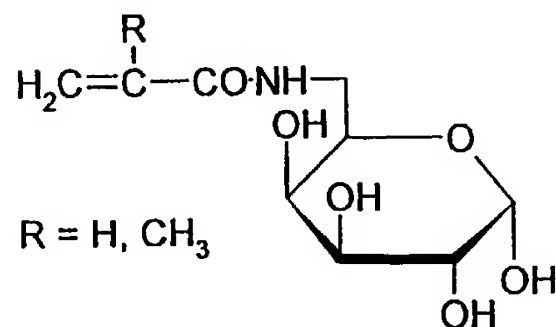
2-(methacryloyloxy)ethyl 2-acetamido-2-deoxy-D-glucopyranoside (for  $n = 1$ )

5



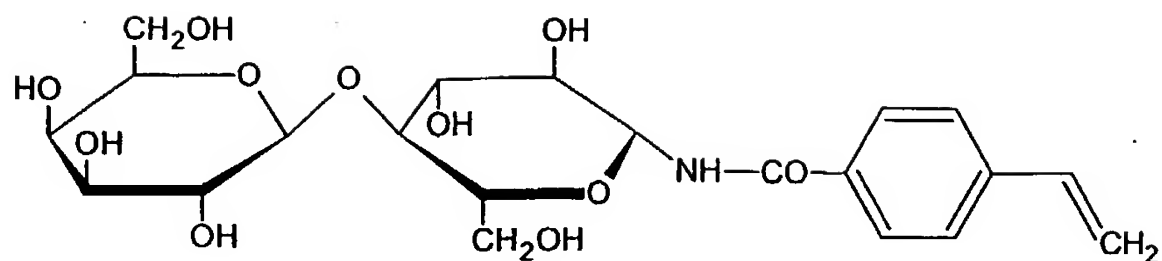
$n$  can be 1-3

6-deoxy-6-methacryloylamino-D-glucopyranose



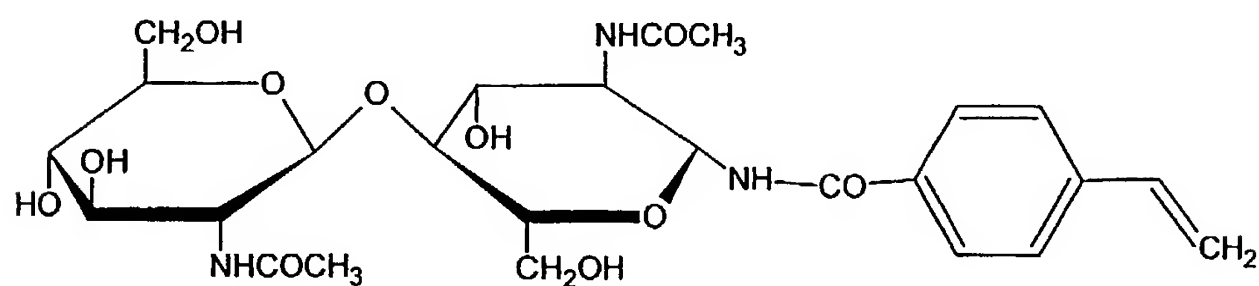
10

4-O-( $\beta$ -D-galactopyranosyl)-N-(4-vinylbenzoyl)(1 $\rightarrow$ 4)- $\beta$ -D-glucopyranosylamine



2-acetamido-4-O-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-deoxy-N-(4-vinylbenzoyl)- $\beta$ -D-glucopyranosylamine

5



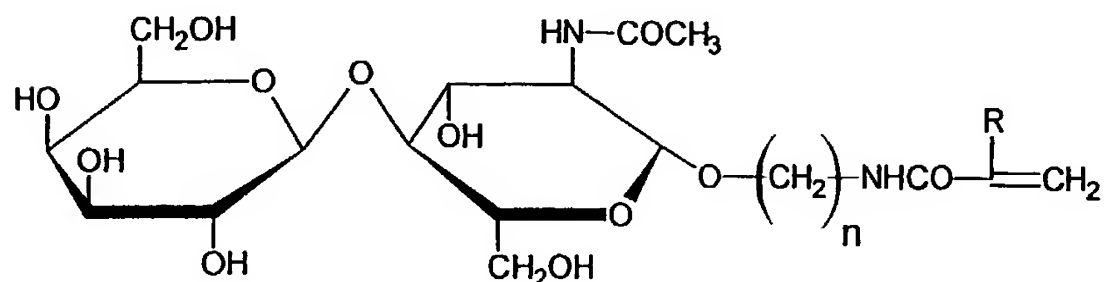
n-(acryloylamino)alkyl O-( $\beta$ -D-galactopyranosyl)(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside, n = 2-10

10

n-(methacryloylamino)alkyl O-( $\beta$ -D-galactopyranosyl)(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside, n = 2-10

2-(acryloylamino)ethyl O-( $\beta$ -D-galactopyranosyl)(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside, n = 2, R = H

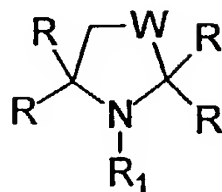
15



R = H, CH<sub>3</sub>

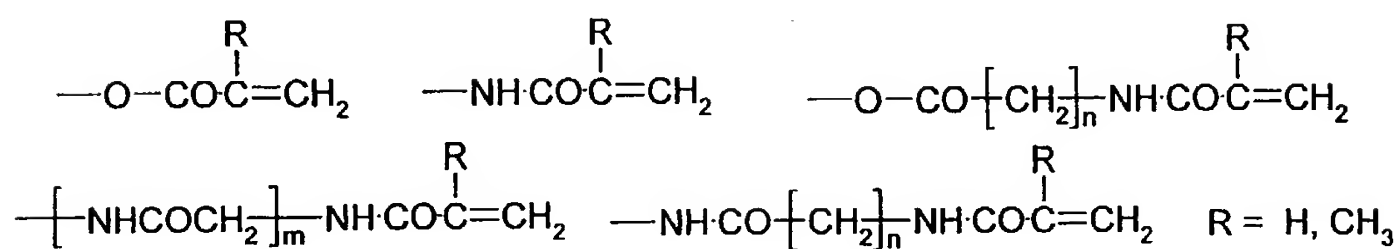
7. A polymer carrier for keratinocyte cultivation as claimed in claims 1-6 wherein it contains, individually or in combination, 0.0-50 wt.-% of the compound selected from the group of polymerizable sterically hindered amine derivatives of general

formula



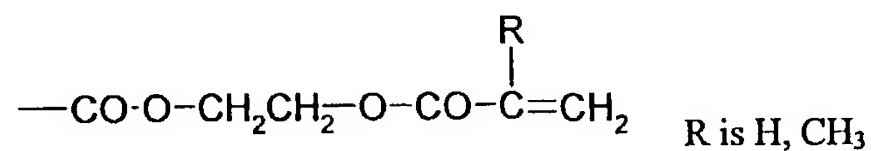
where W is -CH(X)- or -CH(X)CH<sub>2</sub>- and X is a radical-polymerizable group

5



n is 1-10, m is 1 or 2

10

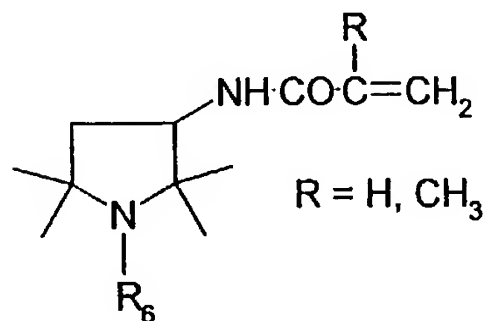


and where R<sub>1</sub> is H or alkyl C<sub>1</sub>-C<sub>4</sub>, R<sub>1</sub> is H or alkyl C<sub>2</sub>-C<sub>4</sub>, OH an oxygen radical formed by additional oxidation, the substances being selected from the group including:

15

*N*-(2,2,5,5-tetramethylpyrrolidin-3-yl)acrylamide

*N*-(2,2,5,5-tetramethylpyrrolidin-3-yl)methacrylamide



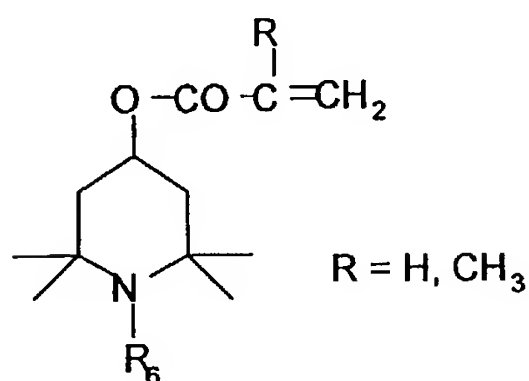
R = H, CH<sub>3</sub>

where R<sub>6</sub> is H or alkyl C<sub>1</sub>-C<sub>4</sub>, OH or an oxygen radical formed by additional oxidation

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(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl) acrylate

(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl) methacrylate

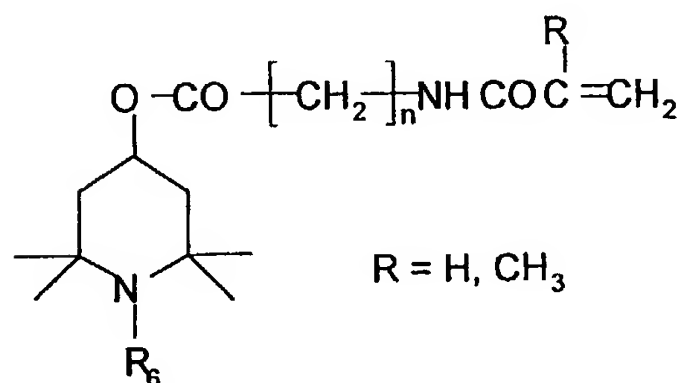


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where  $R_6$  is H or alkyl  $C_1$ - $C_4$ , OH or oxygen radical formed by additional oxidation

(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl) (n+1)-(acryloylamino)alkanoate

10 (1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl) (n+1)-(methacryloylamino)alkanoate  
of general formula

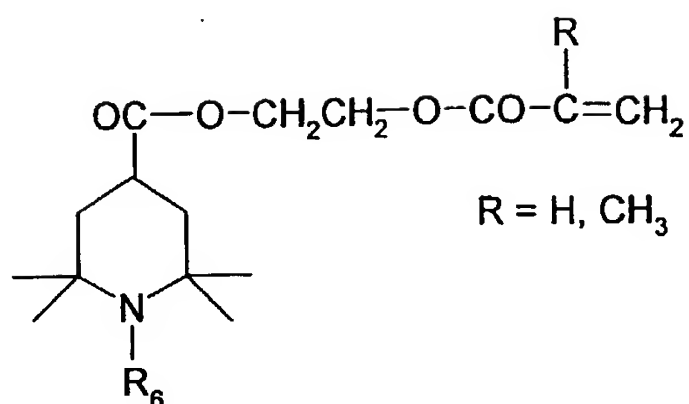


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where  $R_6$  is H or alkyl  $C_1$ - $C_4$ , OH or oxygen radical formed by additional oxidation

2-(acryloyloxy)ethyl 1- $R_6$ -2,2,6,6-tetramethylpiperidine-4-carboxylate

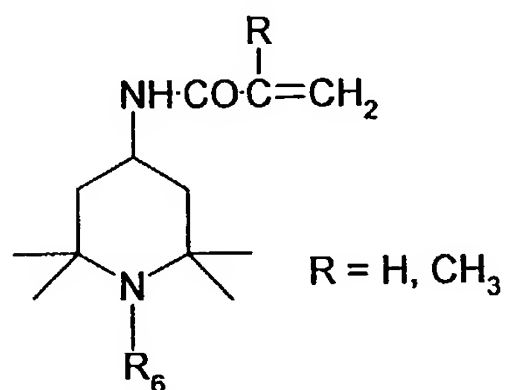
2-(methacryloyloxy)ethyl 1- $R_6$ -2,2,6,6-tetramethylpiperidine-4-carboxylate



where  $R_6$  is H or alkyl  $C_1-C_4$ , OH or oxygen radical formed by additional oxidation

*N*-(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl)acrylamide

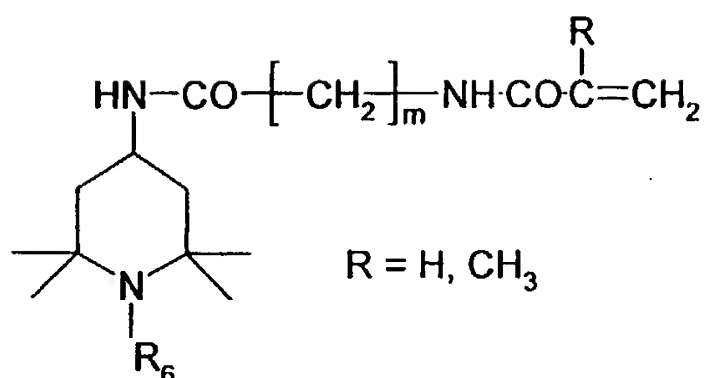
5 *N*-(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl)methacrylamide



where  $R_6$  is H or alkyl  $C_1-C_4$ , OH or oxygen radical formed by additional oxidation

10 *N*-{(m+1)-oxo-(m+1)-[(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl)amino]alkyl}acrylamide

*N*-{(m+1)-oxo-(m+1)-[(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl)amino]alkyl}methacrylamide of general formula:

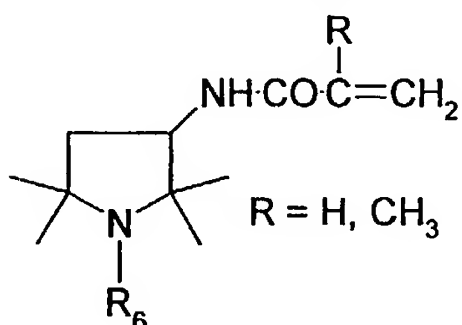


where  $R_6$  is H or alkyl  $C_1$ - $C_4$ , OH or oxygen radical formed by additional oxidation

5

*N*-(1- $R_6$ -2,2,5,5-tetramethylpyrrolidin-3-yl)acrylamide

*N*-(1- $R_6$ -2,2,5,5-tetramethylpyrrolidin-3-yl)methacrylamide



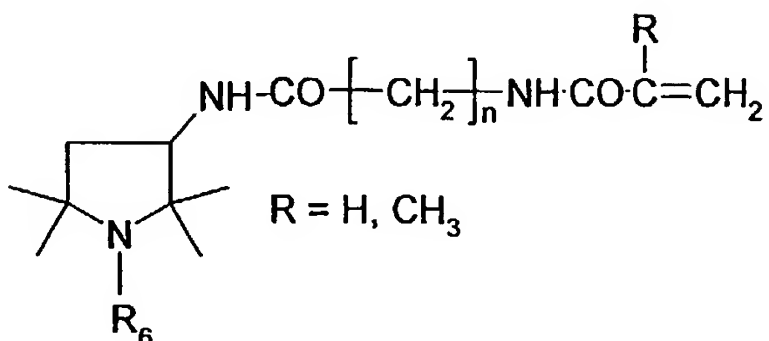
where  $R_6$  is H or alkyl  $C_1$ - $C_4$ , OH or oxygen radical formed by additional oxidation

10

*N*-{(n+1)-oxo-(n+1)-[(1- $R_6$ -2,2,5,5-tetramethylpyrrolidin-3-yl)amino]alkyl}acrylamide

*N*-{(n+1)-oxo-(n+1)-[(1- $R_6$ -2,2,5,5-tetramethylpyrrolidin-3-yl)amino]alkyl}methacrylamide

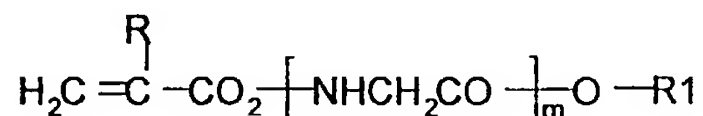
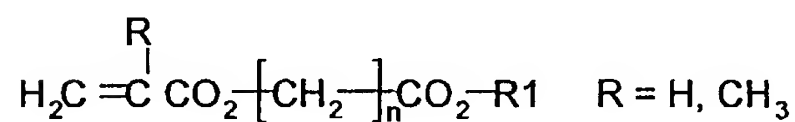
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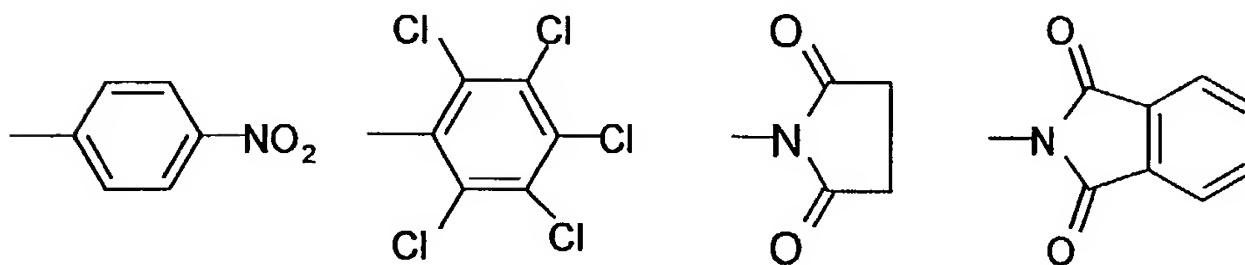
where R<sub>6</sub> is H or alkyl C<sub>1</sub>-C<sub>4</sub>, OH or oxygen radical formed by additional oxidation.

8. A polymer carrier prepared as claimed in claims 1-7 wherein the polymerization mixture contains 0.0-30 wt.-% of polymerizable reactive ω-amino acid derivatives, which are modified in the prepared polymer by the reaction with an appropriate amino derivative of a saccharide or disaccharide, the appropriate ω-amino acid derivatives being:

- a) activated esters of ω-(acryloylamino)alkanoic and ω-(methacryloylamino)alkanoic acids of general formula

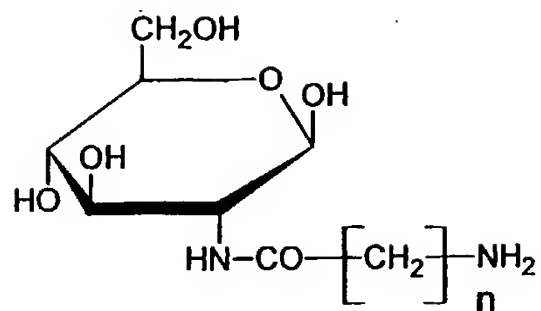


where n = 1-12, m = 2,3, R1 are esters of 4-nitrophenol, pentachlorophenol, N-hydroxysuccinimide, N-hydroxyphthalimide

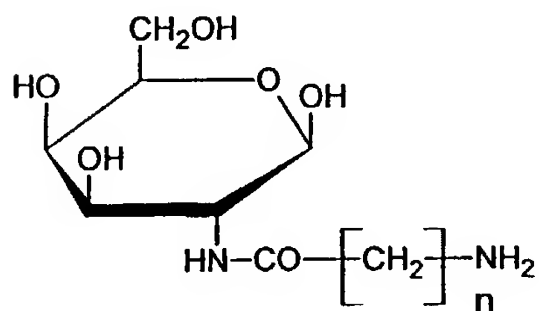


- b) Polymerizable ω-amino acid derivatives, which are activate for the reaction with amino sugars with dicyclohexylcarbodiimide. where R1 is H, the appropriate amino sugar derivatives being

2-[(n+1)-(aminoalkanoyl)amino-2-deoxyglucopyranose for n = 1-12

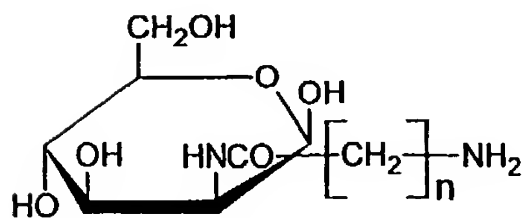


2-[(n+1)-(aminoalkanoyl)amino]-2-deoxygalactopyranose for n = 1-12



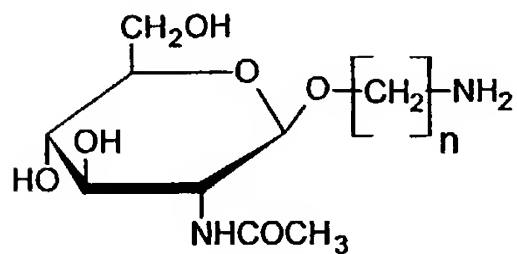
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2-[(n+1)-(aminoalkanoyl)amino]-2-deoxymannopyranose for n = 1-12



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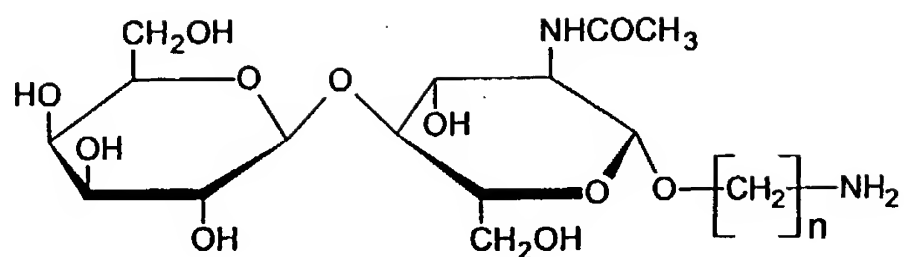
n-aminoalkyl 2-acetamido-2-deoxy-β-D-glucopyranoside, n = 2-10



n-aminoalkyl β-D-galactopyranosyl(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside, x = 2-10

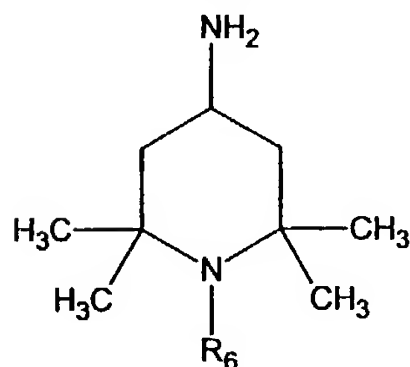
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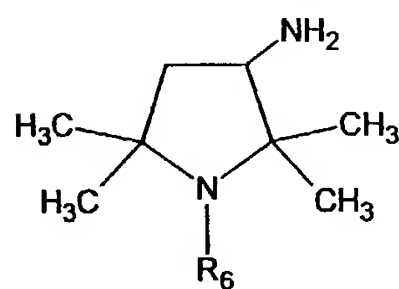
9. A polymer carrier for keratinocyte cultivation prepared as claimed in claims 1-8 wherein the reactive  $\omega$ -amino acid derivatives are additionally modified in the prepared polymer by the reaction with an appropriate amino derivative of a sterically hindered amine, the appropriate sterically hindered amine derivatives being:

4-amino-1- $R_6$ -2,2,6,6-tetramethylpiperidine



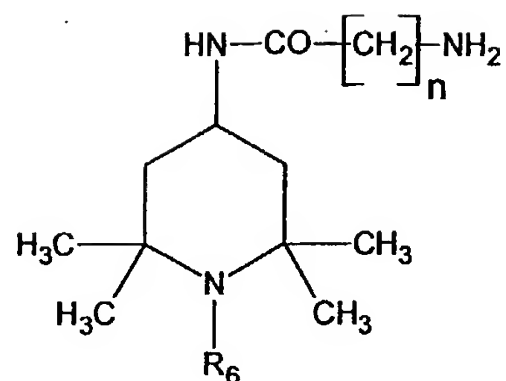
where  $R_6$  is H, alkyl  $C_1$ - $C_4$ , OH or O radical

4-amino-1- $R_6$ -2,2,5,5-tetramethylpyrrolidine



where  $R_6$  is H, alkyl  $C_1$ - $C_4$ , OH or O radical

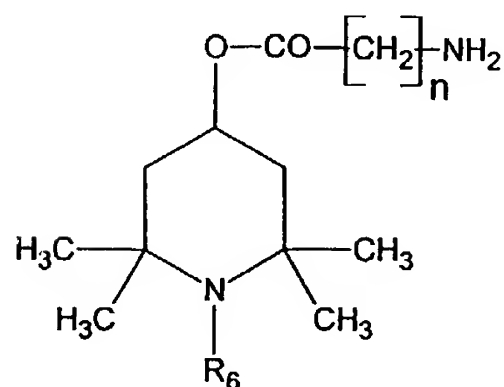
( $n+1$ )-amino- $N$ -(1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl)alkanamide



where  $R_6$  is H, alkyl  $C_1$ - $C_4$ , OH or O radical

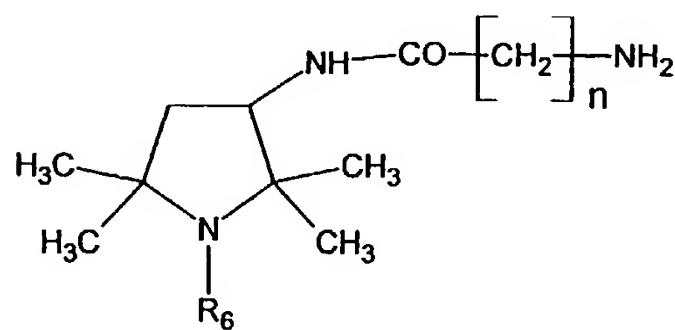
1- $R_6$ -2,2,6,6-tetramethylpiperidin-4-yl (n+1)-aminoalkanoate

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where  $R_6$  is H, alkyl  $C_1$ - $C_4$ , OH or O radical

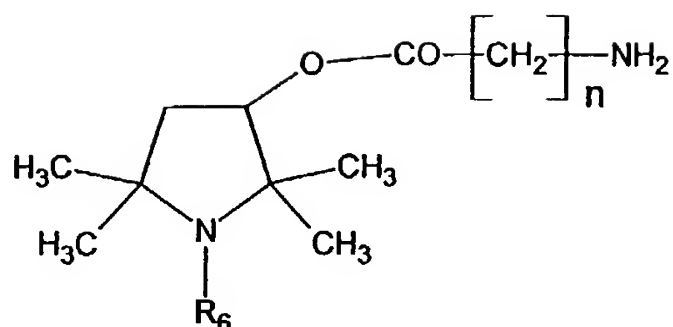
(x+1)-amino-N-(1- $R_6$ -2,2,5,5-tetramethylpyrrolidin-3-yl)alkanamide



10

where  $R_6$  is H, alkyl  $C_1$ - $C_4$ , OH or O radical

1- $R_6$ -2,2,5,5-tetramethylpyrrolidin-3-yl (n+1)-aminoalkanoate



where  $R_6$  is H, alkyl  $C_1$ - $C_4$ , OH or O radical.

- 5      10. A polymer carrier for keratinocyte preparation claimed in claims 1-9 wherein  
 it is prepared by conditioning of the polymer carrier by adsorption of biologically  
 active saccharides selected from the group of polysaccharides heparin, heparan  
 sulfate, hyaluronic acid, monosaccharides conjugated with albumin or polymer  
 carrier of glucuronic acid,  $\beta$ -D-galactose,  $\beta(\alpha)$ -D-N-acetylgalactosamine,  $\beta(\alpha)$ -  
 10 D-N-acetylglucosamine,  $\alpha$ -D-mannose, where the adsorption proceeds at  
 concentrations 10-500  $\mu\text{g/ml}$  PBS at 4-37° C for 1-12 h.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CZ 99/00017

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N5/00 C08F220/58 C08F220/60 C08F220/26 C08F220/34

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C08F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 08448 A (BAY MICHAEL) 3 November 1988 (1988-11-03) page 10, line 10 -page 11, line 20 page 12, line 6-13 ---	1-4
X	EP 0 387 975 A (GRACE W R & CO) 19 September 1990 (1990-09-19) page 5, line 3 -page 7, line 11 ---	1-4
X	US 5 173 421 A (KINIWA HIDEAKI ET AL) 22 December 1992 (1992-12-22) column 3, line 67 -column 4, line 52; claims 1,5,7-9 ---	1-4
X	WO 95 32743 A (USTAV MAKROMOLEKULARNI CHEMIE ;LEKARSKA FAKULTA UNIVERZITY KA (CZ)) 7 December 1995 (1995-12-07) claims 1-3 ---	1,2
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

27 September 1999

Date of mailing of the international search report

06/10/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Meulemans, R

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/CZ 99/00017

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 425 601 A (BIOCARB AB) 8 May 1991 (1991-05-08) the whole document -----	6

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CZ 99/00017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8808448 A	03-11-1988	AU 614747 B AU 1685288 A DK 521989 A EP 0355112 A JP 2504221 T	12-09-1991 02-12-1988 05-12-1989 28-02-1990 06-12-1990
EP 0387975 A	19-09-1990	AU 621722 B AU 5128990 A CA 2012309 A JP 3266980 A	19-03-1992 27-09-1990 16-09-1990 27-11-1991
US 5173421 A	22-12-1992	JP 2291260 A JP 2163077 A EP 0373626 A	03-12-1990 22-06-1990 20-06-1990
WO 9532743 A	07-12-1995	CZ 9401314 A	17-04-1996
EP 0425601 A	08-05-1991	SE 463314 B DE 69027397 D DE 69027397 T AT 139239 T AU 5188490 A CA 2028094 A JP 4500092 T SE 8900605 A WO 9010023 A US 5162471 A	05-11-1990 18-07-1996 23-01-1997 15-06-1996 26-09-1990 02-09-1990 09-01-1992 02-09-1990 07-09-1990 10-11-1992